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Note

A new limonoid from *Aphanamixis polystachya*

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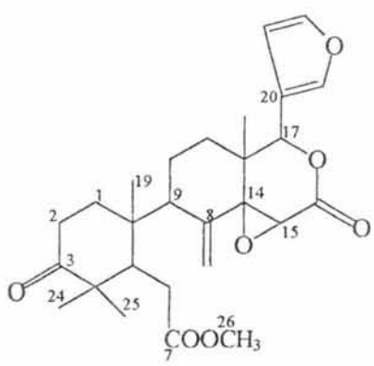
A new compound isolated from the bark of the *Aphanamixis polystachya* has been characterised as dihydroamoorinin **2** by physico-chemical studies.

Aphanamixis polystachya (Syn. *Amoora rohituca*) commonly known as Rohituka (Meliaceae) is a genus of evergreen trees found abundantly in India mainly in the sub-Himalyan tracts, Bengal, Sikkim, Assam, western ghats and Andamans. All the parts of this plant viz. bark, fruits, leaves taste bitter and is widely used in folk medicine. The bark is strongly astringent and is used in diseases of the liver, the spleen, for tumors and abdominal complaints. The pounded bark is also used in rheumatism[1-3]. The dark brown stem bark of the plant is known to contain aphanamixinin[4], β-sitosterol, stigmasterol, aglaiol-3-*O*-rhamnosyl-xyloside[5], 1,5-dihydroxy-6,7,8–trimethoxy-2-methyl-anthraquin-one-3-*O*-β-D-xylopyranoside, naringenin-7,4′-dimethylether-5-*O*-α-L-rhamnopyranoside[6] and amoorinin[7]. In search of other minor constituents, the stem bark extract of *A. polystachya* was chemically reinvestigated which resulted in a new tetranortriterpene limonoid dihydroamoorinin. Its structure was determined with the help of spectroscopic data and chemical correlation.

The chloroform extract of bark of *A. polystachya* on repeated silica gel chromatography yielded a new compound **2** and known compounds aphanamixinin **1** and amoorinin **3**. The compound **2** analysed for $C_{27}H_{36}O_7$ as indicated by its molecular ion at m/z 472 in its EIMS and elemental analysis. The IR spectra showed the characteristic peaks at 3450 (broad) for a secondary hydroxyl group, a sharp single peak at 1728 for δ-lactone, at 1725 for ester, at 1276 for epoxide, two peaks at 1150 and 873 characteristic for furan ring and at 906 cm^{-1} for exocyclic methylene groups.

The ^1H NMR spectrum suggested that compound **2** is closely related to amoorinin **3** and exhibited four singlets at δ 0.95, 1.00, 1.08 and 1.10 assigned to the four tertiary methyl groups; a double doublet at δ 3.61 (J=4.00 and 12.0 Hz) showed the presence of β-OH group at C-3 position. Its C-3 carbon appeared at δ 77.5 in its ^{13}C NMR spectrum[7]. The presence of a sharp singlet at δ 3.75 (3H) indicated the presence of a carbomethoxy group at C-7. This observation suggested a B seco ring structure[8] having a $\Delta^{8(27)}$ double bond which is further supported by two proton resonances at δ 4.90 and 5.10.



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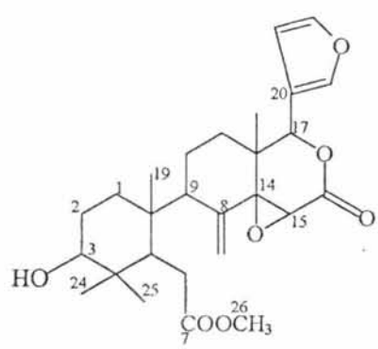

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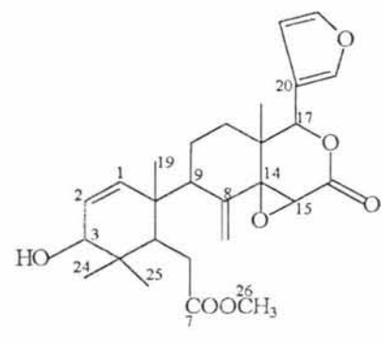

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The typical Ekebergin type ring D was indicated by C-16 lactone resonance at δ 169.0 in the ^{13}C NMR spectrum. Singlet (one proton each) at δ 5.65 and δ 3.8 were observed for C-17β-methine and C-15α-epoxymethine protons. The corresponding carbon resonances occur at δ 79.9 and 50.25 respectively.

In ^{1}H NMR, the β-substituted furan ring resonances appeared at δ 7.39 (H-21), 7.20 (H-23), 6.28 (H-22) corresponding to 2α furano protons and 1β furano proton. The ^{13}C NMR spectrum supported the carbon resonances appearing at δ 143.0, 141.10, and 110.3; and at δ 121.0 for C-20 carbons[4, 8]. All the above described data indicated that structure of compound **2** is similar to **3**, except the double bond at $\Delta^{1(2)}$.

Acetylation of compound **2** with Ac_2O and dry pyridine yielded a monoacetate **2a** as indicated by the appearance of a new methyl singlet at δ 2.10 in its ^{1}H NMR spectra. The oxidation of **2** with CrO_3-pyridine complex gave the known 3-keto derivative **1** (co-TLC, mmp, IR, ^{1}H NMR, EIMS). Thus the structure 2 was established as dihydroamoorinin.

Experimental Section

Melting points are uncorrected. IR spectra were recorded in KBr. The 200 MHz ^{1}H NMR spectra of **1, 2** and **3** were obtained in $CDCl_3$ with TMS as internal standard. ^{13}C NMR spectra were determined in $CDCl_3$ at 50 MHz. EI-MS were recorded at 70 eV. Column chromatography, vacuum funnel chromatography and TLC were done on silica gel (Ranbaxy, 60-120 mesh), silica gel H without binder and silica gel G respectively. Detection of spots was carried out by exposure to I_2 vapours and / or by spraying with 5 % vanillin-sulphuric acid solution followed by heating at 105° for 5 min. The stem bark of the *A. polystachya* were purchased from the local market and identified in our Botany Department, where a voucher specimen has been maintained.

Extraction and isolation of compounds. The air dried and powdered stem bark (3.4 kg) was extracted with MeOH (3 × 6.0 L), in a perculator at room temperature. After filtration, the dark brown methanolic extract was evaporated to dryness under reduced pressure below 60° to get a dark brown mass (232 g) which was dissolved in MeOH-water (In 3:1 ratio, 1 L). This aq. methanolic extract was consecutively extracted thrice with $CHCl_3$ (97 g) and EtOAc (86.8 g).

The $CHCl_3$ fraction (97 g) was chromatographed in a sintered funnel over silica gel H (500 g) *in vacuo* and eluted with hexane, hexane-$CHCl_3$ (1:1), $CHCl_3$ and $CHCl_3$-MeOH [1, 3, 5, 10, 20, 30 and 50%] and MeOH successively. The eluates were monitored by TLC and grouped into 11 fractions.

Fraction No. 2 (3.5 g) on silica gel rechromatography using hexane- $CHCl_3$ mixtures as eluate yielded compound **1** (200 mg).

The fraction No. 3 (11 g) eluted with $CHCl_3$ was rechromatographed on silica gel column (220 g) using hexane, hexane-$CHCl_3$ (1:2), $CHCl_3$ and increasing proportions of $CHCl_3$-MeOH yielding compounds **2** (50 mg) and **3** (265 mg).

Compound 2 (Dihydroamoorinin) : Colourless needles, mp 197-98°; R_f : 0.4 (hexane-EtOAc=2:1); IR : 3450 (-OH), 1728 (δ-lactone), 1725 (ester), 1570, 873 (furan ring), 1276 (epoxide), 906 (exocyclic methylene); EIMS m/z (rel. int.) : 472 $[M]^+$ (4.5), 470 (100), 413 (4.3), 405 (3), 214 (3), 189 (2.4), 67 (16.6), 59 (3); ^{1}H NMR : δ 3.61 (1H, dd, **J**=4, 12 Hz, H-3), 3.80 (1H, s, H-15), 5.65 (1H, s, H-17), 1.08 (3H, s, H-18), 1.10 (3H, s, H-19), 7.39 (1H, s, H-21), 6.28 (1H, s, H-22), 7.20 (1H, s, H-23), 0.95 (3H, s, H-24), 1.00 (3H, s, H-25), 3.75 (3H, s, H-26), 4.90 (1H, s, H-27a), 5.10 (1H, s, H-27b); ^{13}C NMR : δ 39.7 (C-1), 29.6 (C-2), 77.5 (C-3), 41.8 (C-4), 43.2 (C-5), 34.3 (C-6), 174.0 (C-7), 146.0 (C-8), 50.2 (C-9), 44.4 (C-10), 24.1 (C-11), 21.0 (C-12), 48.4 (C-13), 80.5 (C-14), 50.25 (C-15), 169.0 (C-16), 79.9 (C-17), 26.2 (C-18), 14.1 (C-19), 121.0 (C-20), 143.1 (C-21), 110.3 (C-22), 141.1 (C-23), 21.8 (C-24), 22.0 (C-25), 52.4 (C-26), 111.9 (C-27); Anal. Found : C, 68.52 ; H, 7.44. Calc. for $C_{27}H_{36}O_7$:C, 68.64 ; H, 7.63 %.

Mono aceState of 2 : Compound **2** (5 mg) was dissolved in Ac_2O (0.5 mL) and C_5H_5N (0.5 mL) and left to stand overnight. Usual work-up yielded a colourless amorphous compound **2a.** IR : 1730 (δ-lactone), 1737 (OAc), 1723 (ester), 1570, 873 (furan ring), 1272 (epoxide), 910 (exocyclic methylene) cm^{-1}; ^{1}H NMR: δ 2.1 (3H, s, OAc) and other proton signals.

CrO_3 oxidation of 2. Compound **2** (10 mg) dissolved in C_5H_5N (0.5 mL) and CrO_3-C_5H_5N (10 mg, dried *in vacuo* over P_2O_5) slurry was mixed together with stirring at 0-5° for 6 hr. The reaction mixture was worked-up as usual. The product crystallised as prisms, which was found to be identical with aphanamixinin (**1**, 5.2 mg, co-TLC, mmp 208-10°, IR, ^{1}H NMR, EIMS).

Acknowledgement

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